American Journal of Hematology 55:183–187 (1997)

Increased Tissue Factor Pathway Inhibitor in Patients With Acute Myocardial Infarction

Yuko Kamikura,[1] Hideo Wada,[1]* Atsushi Yamada,[1] Minori Shimura,[1] Katsuyo Hiyoyama,[1] Hiroshi Shiku,[1] Motoaki Tanigawa,[2] Hideo Nishikawa,[2] Norikazu Yamada,[3] Naoki Isaka,[3] Takeshi Nakano,[3] Kousuke Kumeda,[4] and Hisao Kato[5]

[1]Second Department of Internal Medicine, Mie University School of Medicine, Tsu-city, Japan
[2]Department of Internal Medicine, Yamada Red Cross Hospital, Watarai-gun, Japan
[3]First Department of Internal Medicine, Mie University School of Medicine, Tsu-city, Japan
[4]Chemo-Sero-Therapeutic Research Institute, Kumamoto-city, Japan
[5]National Cardiovascular Center Research Institute, Suita-city, Japan

We examined hemostatic abnormalities in 23 patients with acute myocardial infarction (AMI), 10 with pulmonary embolism (PE), and 10 with deep vein thrombosis (DVT). At the onset of AMI, plasma levels of tissue-type plasminogen activator (t-PA), PA inhibitor-I (PAI-I), fibrin-D-dimer, thrombin-antithrombin complex (TAT), and plasmin-plasmin inhibitor complex (PPIC) were significantly increased. Both the plasma total TFPI and free-TFPI levels in the AMI patients were significantly higher than those in the healthy volunteers, PE patients, and DVT patients. There was no significant difference in total TFPI or free-TFPI among patients with PE, those with DVT, and healthy volunteers. One hour after percutaneous transluminal coronary angioplasty (PTCA) in the AMI group, the total TFPI level was further increased, and it was significantly reduced 24 hr after PTCA, to a level similar to that in healthy volunteers. Free-TFPI showed a pattern similar to that of total TFPI. The ratio of free-TFPI/total TFPI was highest 1 hr after PTCA. Increased TFPI in AMI patients might be released from ischemic tissues. Am. J. Hematol. 55:183–187, 1997.

Key words: PTCA; AMI; TFPI; free-TFPI

INTRODUCTION

Acute myocardial infarction (AMI) can be fatal; however, survival and cardiovascular morbidity are improved by thrombolytic therapy or percutaneous transluminal coronary angioplasty (PTCA) at onset. Hypercoagulability may be involved in the pathogenesis of AMI, and in the reocclusion of the coronary artery after PTCA. Coronary thrombosis generally occurs at sites of stenosis, often precipitated by fissuring of an atherosclerotic plaque [1]. Since plaques contain tissue factor (TF)-synthesizing cells, plaque rupture leads to exposure of TF activity [2] and activation of the TF pathway, the consequences of which are thrombin formation, platelet activation, and fibrin deposition. Elevated plasma TF pathway inhibitor (TFPI) activity and Factor VII have been demonstrated in patients with AMI [3,4]. TFPI contains an acidic amino-terminal domain followed by three tandem Kunitz-type protease inhibitory domains (K1, K2, and K3) and a basic carboxy-terminal domain [5]. TFPI inhibits Factor Xa directly, and also inhibits the proteolytic activity of the F VIIa/TF complex by forming a quaternary complex: F Xa/TFPI/F VIIa/TF. TFPI is synthesized mainly by endothelial cells, and exists on the endothelium and in plasma in vivo [6,7]. In plasma, there is a free form of TFPI, and lipoprotein-associated forms of TFPI. The level of plasma TFPI and the proportion of each form of TFPI varies in several pathophysiological conditions [8–11]. The C-terminal basic region of TFPI is essential for binding with low-density lipoprotein (LDL) and very-

Contract grant sponsor: Ministry of Education, Science and Culture, Japan.

*Correspondence to: Hideo Wada, MD, The 2nd Department of Internal Medicine, Mie University School of Medicine, 2-174 Edobashi, Tsu-city, Mie-ken 514, Japan.

Received 5 August 1996; Accepted 12 February 1997.

TABLE I. Hemostatic Abnormalities in Patients With AMI, PE, and DVT, and in Healthy (Normal) Volunteers

		Normal	AMI	PE	DVT
APTT	(sec)	31.5 ± 2.3	33.4 ± 8.6	33.5 ± 10.2	32.9 ± 6.4
PT	(sec)	11.3 ± 0.7	12.6 ± 1.4	12.9 ± 1.7	12.4 ± 1.7
Fibrinogen	(mg/dl)	205.4 ± 12.9	291.2 ± 68.5**	285.4 ± 73.2	264.1 ± 77.5
Antithrombin	(%)	97.6 ± 9.5	93.6 ± 10.4	98.4 ± 12.7	101.2 ± 19.6
Protein C; Ac	(%)	104.5 ± 10.8	78.0 ± 15.3	78.9 ± 21.4	88.6 ± 19.1
Protein S; Ag	(%)	96.7 ± 8.4	93.9 ± 9.90	99.6 ± 16.4	95.4 ± 11.7
TAT	(ng/ml)	1.65 ± 0.45	12.41 ± 4.25*	13.72 ± 10.56*	6.72 ± 3.49*
PPIC	(ng/ml)	0.44 ± 0.18	0.84 ± 0.27**	1.56 ± 1.21**	0.99 ± 0.73**
Fibrin-D-dimer	(ng/ml)	49.5 ± 11.7	194.7 ± 82.1*	164.3 ± 137.2*	109.3 ± 57.5*
t-PA	(ng/ml)	4.54 ± 0.93	21.60 ± 9.43***	10.77 ± 5.94**	9.74 ± 3.31**
PAI-I	(ng/ml)	5.8 ± 1.6	26.01 ± 14.96*	21.9 ± 8.4*	38.90 ± 19.70**

*$P < 0.01$ compared with normal.
**$P < 0.05$ compared with normal.
***$P < 0.01$ compared with PE or DVT.
****$P < 0.05$ compared with PE or DVT.

low-density lipoprotein (VLDL) [12]. In the present study, we measured plasma free-TFPI and total TFPI using a monoclonal antibody against rTFPI and a polyclonal antibody. The epitope of the monoclonal antibody was found to be the K3 domain, and only the free form TFPI in plasma, not the lipoprotein-associated form, was detected by enzyme immunoassay (EIA) [13,14].

MATERIALS AND METHODS

Over the past 2 years, we have examined hemostatic abnormalities in 23 consecutive AMI patients (17 males and 6 females) who received PTCA. None of the patients had severe liver dysfunction or congenital thrombophilia. The mean age of the patients was 51.1 ± 7.8 years, and the mean level of total cholesterol was 244 ± 75 mg/dl. Thirty healthy volunteers (mean age 30.4 ± 7.6 years, 10 females and 20 males), 10 patients with deep vein thrombosis (DVT) (mean age 45.4 ± 19.7 years, 6 females and 4 males), and 10 patients with pulmonary embolism (PE) (mean age 58.4 ± 24.6 years, 5 females and 5 males) were also examined. After informed consent was obtained from each participant in the study, coronary arteriography was performed. After blood sampling, 5,000 U of heparin was injected intravenously, and 24,000 U/day of heparin was continued. The infarct-related vessel was defined, and PTCA was then performed using an 8F thin-walled guiding catheter and a low-profile balloon catheter. Blood specimens were collected before PTCA and the first injection of heparin, 1 hr and 24 hr after PTCA, and 3 weeks after PTCA.

For the assay of TFPI, whole blood was anticoagulated by the addition of 9 volumes of blood to 1 volume of 3.8% trisodium citrate solution. For the determination of fibrin-D-dimer, the whole blood was anticoagulated by the addition of 9 volumes of blood to 1 volume of 3.8% trisodium citrate supplemented with 1,000 U/ml heparin and 1,000 KIE/ml aprotinin.

Plasma antithrombin and protein C activity were measured by an amidolytic assay, using Berichrom AT III and Berichrom-Protein C (Behringwerke AG). Plasma protein S antigen was measured by enzyme-linked immunosorbent assay (ELISA), using anti-protein S polyclonal antibody (Dakopatts, Glostrup, Denmark). Thrombin-antithrombin complex (TAT), plasmin-plasmin inhibitor complex (PPIC), fibrin-D-dimer, tissue type plasminogen activator (t-PA), and plasminogen activator inhibitor-I (PAI-I) antigens were determined with Enzygnost-TAT (Behringwerke AG), PPIC-test (Teijin), Frelisa D-dimer (Agen), one-step sandwich ELISA t-PA (Fuzirebio), and Imulyse™ PAI-I (Biopool), respectively.

Free-TFPI antigen levels were measured by a one-step sandwich ELISA kit (Chemo-Sero-Therapeutic Research Institute), using two different monoclonal antibodies against TFPI obtained by the cell fusion method [13,14]. One monoclonal antibody, which immobilized to the solid phase (microplate well), recognized the K3 domain [13], and the other monoclonal antibody, which conjugated with horse radish peroxidase (HRP), recognized the specific conformation formed between the K1 and K2 domains [14]. The interassay and intraassay coefficients of variation of this kit at the free-TFPI antigen level of 12.5 ng/ml were 4.2 and 5.6%, respectively.

Total-TFPI antigen levels were measured by a one-step sandwich ELISA kit (Chemo-Sero-Therapeutic Research Institute) with polyclonal antibody and monoclonal antibody [15]. The rabbit anti-TFPI polyclonal antibody was immobilized to the solid phase (microplate well), and the monoclonal antibody, which conjugated with HRP, recognized the specific conformation formed between the K1 and K2 domains. The interassay and intraassay coefficients of variation of this kit at the total TFPI antigen level of 12.5 ng/ml were 3.5 and 5.3%, respectively.

The results are expressed as the mean ± one standard

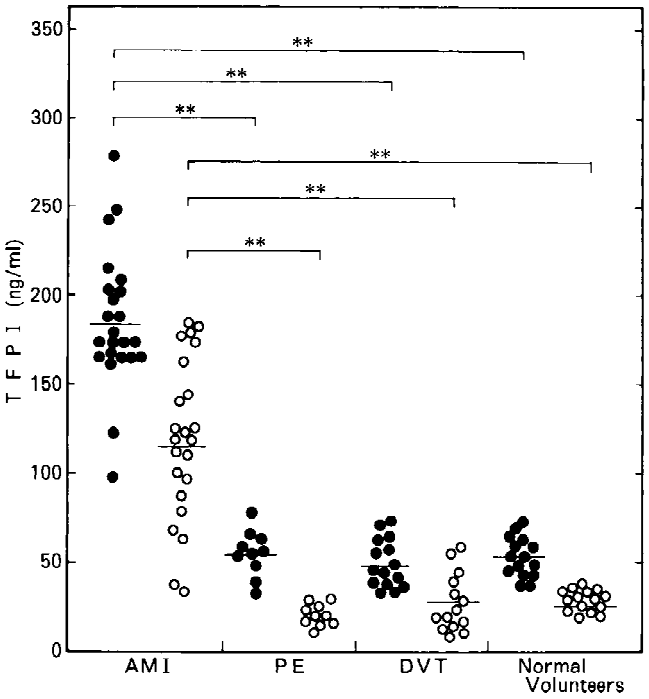

Fig. 1. Plasma TFPI levels in AMI, PE, and DVT patients at onset and in healthy volunteers. Closed circles: total TFPI; open circles: free TFPI. **$P < 0.01$.

deviation (SD). Statistical analysis was performed with the Mann-Whitney U-test and paired Wilcoxon test. Probability values less than 0.05 were accepted as significant.

RESULTS

At the onset of AMI, the activated partial thromboplastin time (APTT), prothrombin time (PT), fibrinogen level, antithrombin activity, antiplasmin activity, plasminogen activity, protein C activity, and protein S antigen levels of all of the patients were within the normal ranges. The plasma levels of TAT, fibrin-D-dimer, t-PA, PAI-I, and fibrin-D-dimer in the patients with AMI were significantly increased, and the plasma t-PA level in the AMI patients was significantly higher than that in the patients with PE or DVT (Table I). Both plasma total TFPI (183.5 ± 49.0 ng/ml) and free TFPI (117.6 ± 44.5 ng/ml) levels in the AMI patients were significantly higher than those in the healthy volunteers (52.4 ± 9.9 ng/ml and 25.1 ± 4.8 ng/ml, $P < 0.01$ and $P < 0.01$, respectively). There was no significant difference in total TFPI or free TFPI among the patients with PE, those with DVT, and healthy volunteers (Fig. 1). One hour after PTCA, the total TFPI level (231.0 ± 33.7 ng/ml) in the AMI group was further increased, and it was significantly reduced 24 hr after PTCA (86.7 ± 11.2 ng/ml) compared to before PTCA ($P < 0.01$). Three weeks after

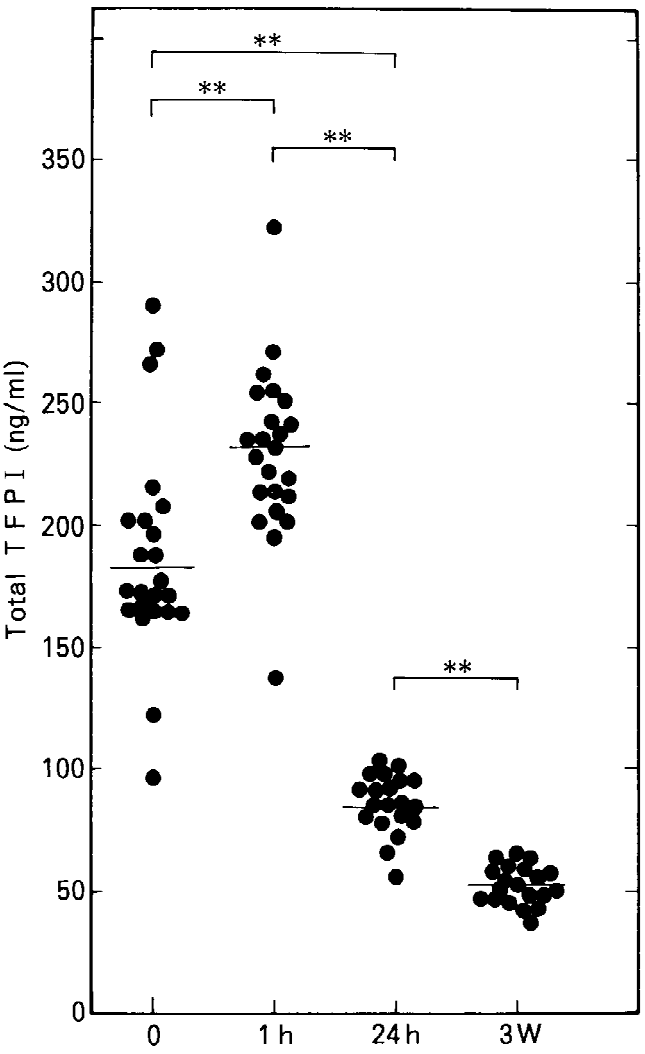

Fig. 2. Plasma total TFPI levels in AMI patients after PTCA. o, before PTCA; 1 h, 1 hr after PTCA; 24 h, 24 hr after PTCA; 3 W, 3 weeks after PTCA. **$P < 0.01$.

PTCA, the total TFPI level was similar to that in healthy volunteers (Fig. 2). Free-TFPI had a pattern similar to that of total TFPI (Fig. 3). The ratio of free-TFPI/total TFPI was highest 1 hr after PTCA (Fig. 4). One hour after PTCA, the t-PA and PPIC levels were also increased in the AMI patients and reduced 3 weeks after PTCA (Table II).

DISCUSSION

TFPI is a Kunitz-type protease inhibitor with three tandem inhibitory domains. TFPI activity is distributed among LDL/VLDL-associated, high density-lipoprotein (HDL)-associated, and free forms of TFPI after gel filtration of human plasma, and the free-form TFPI activity was observed to be decreased in hyperlipidemic patients [16].

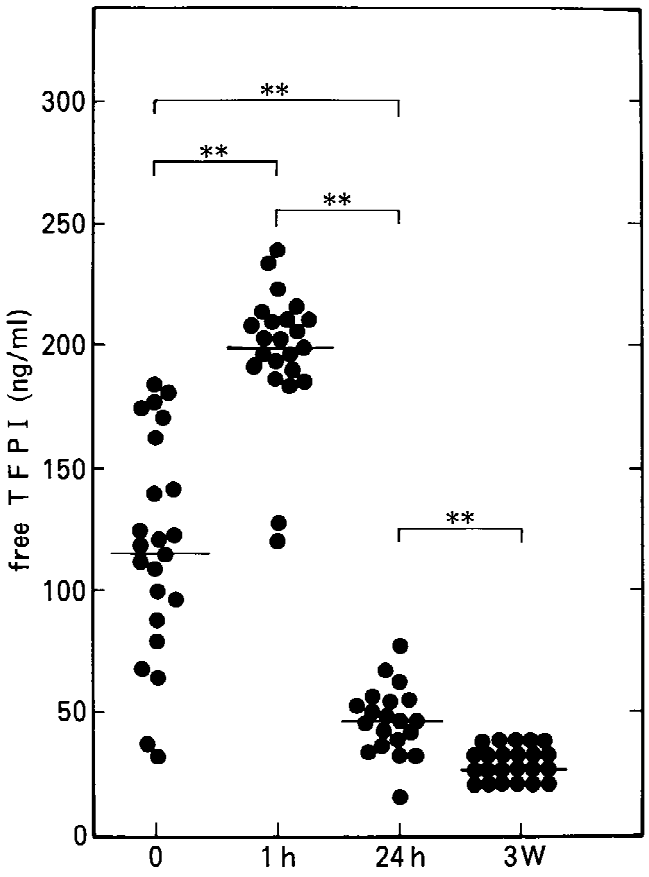

Fig. 3. Plasma free-TFPI levels in AMI patients after PTCA. o, before PTCA; 1 h, 1 hr after PTCA; 24 h, 24 hr after PTCA; 3 W, 3 weeks after PTCA. **$P < 0.01$.

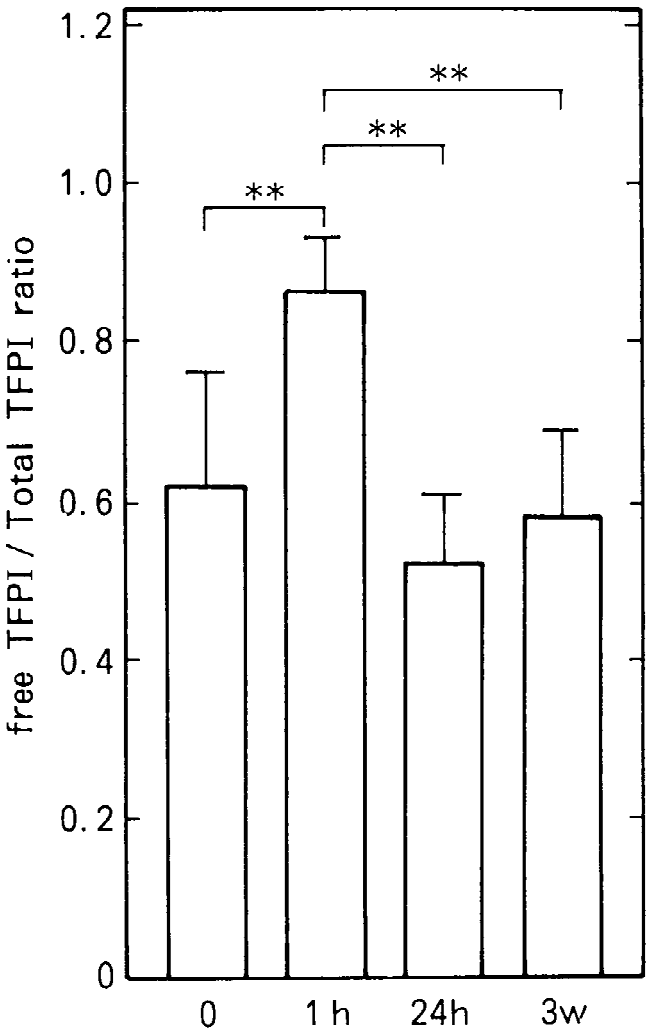

Fig. 4. The ratio of free-TFPI/total-TFPI in AMI patients after PTCA. o, before PTCA; 1 h, 1 hr after PTCA; 24 h, 24 hr after PTCA; 3 W, 3 weeks after PTCA. **$P < 0.01$.

At the onset of AMI in the present series of patients, the screening results of hemostasis were within normal range. Since AMI is a small and local thrombosis, the hemostatic abnormalities in AMI are less than those in disseminated intravascular coagulation (DIC) [17], although some cases of AMI are fatal. The hemostatic molecular markers such as TAT, PPIC, t-PA, PAI-I, and D-dimer in our AMI patients were significantly increased. These markers were sensitive and specific for thrombosis. Both plasma total TFPI and free-TFPI levels in the AMI patients were significantly increased, and these levels were not increased in the patients with PE or DVT. These findings suggest that the increase of TFPI may not be caused by thrombosis. Although the abnormalities of TAT, PPIC, PAI-I, and fibrin-D-dimer in the AMI patients were similar to those in the DVT and PE patients, the plasma t-PA level in the AMI patients was significantly higher than that in the DVT and PE patients. Plasma t-PA is released from vascular endothelial cells. Increased plasma TFPI was reported in DIC [18], and severe sepsis [10]. Our present data thus suggest that the plasma TFPI level increases in arterial thrombosis or ischemic change, and does not increase promptly in venous thrombosis. The increase of TFPI might be released from ischemic tissue.

The administration of heparin increases the plasma TFPI level [19]. In our study, heparin was not injected until the first blood sampling. One hour after PTCA, plasma TFPI levels were further increased, and the ratio of free-TFPI/total TFPI was at its highest, suggesting that increased TFPI 1 hr after PTCA was released from vascular endothelial cells. One possible explanation for this result is the release of TFPI from vascular endothelial cells caused by heparin injection. Another possibility is the release caused by tissue injuries by PTCA, because plasma t-PA released from vascular endothelial cells was markedly increased 1 hr after PTCA. Plasma TFPI was significantly reduced 24 hr after PTCA, when its level became similar to that of the healthy volunteers, suggesting that the increase of plasma TFPI level at the onset of AMI was not caused by congenital disease. Since the

TABLE II. Hemostatic Abnormalities in AMI Patients During PTCA

		Time after PTCA			
		0	1 hr	24 hr	3 wk
TAT	(ng/ml)	12.41 ± 4.25	11.26 ± 3.12	11.71 ± 8.89	4.26 ± 1.32*
PPIC	(ng/ml)	0.84 ± 0.27	2.30 ± 0.70*	0.70 ± 0.17	0.40 ± 0.10**
Fibrin-D-dimer	(ng/ml)	194.7 ± 82.1	233.0 ± 87.9	199.9 ± 94.3	77.3 ± 45.0*
t-PA	(ng/ml)	21.60 ± 9.43	31.05 ± 8.46*	36.96 ± 6.9*	13.44 ± 3.96**
PAI-I	(ng/ml)	26.01 ± 14.96	28.24 ± 15.02	28.78 ± 12.77	25.49 ± 12.80

*$P < 0.01$.
**$P < 0.05$.

free-TFPI level was also increased in the AMI patients at onset, it is thought that the increase of TFPI level in the AMI patients was not caused by hyperlipidemia.

In conclusion, the measurement of both total TFPI and free-TFPI might be useful for the examination of pathological conditions in AMI.

ACKNOWLEDGMENTS

This work was supported in part by a Grant-in-Aid for Cancer Research from the Ministry of Education, Science and Culture, Japan.

REFERENCES

1. Davies MJ, Woolf N: Atherosclerosis. What is it and why does it occur? Br Heart J 69(Suppl):S3–S11, 1993.
2. Wilcox JN, Smith KM, Schwartz SM, Gordon D: Localization of tissue factor in the normal vessel wall and in the atherosclerotic olaque. Proc Natl Acad Sci USA 86:2639–2643, 1989.
3. Sandest PM, Sirnes PA, Abildgaard U: Factor VII and extrinsic pathway inhibitor in acute coronary disease. Br J Haematol 72:391–396, 1989.
4. Moor E, Hamsten A, Karpe F, Bavenholm P, Blomback M, Silveria A: Relationship of tissue factor pathway inhibitor activity to plasma lipoproteins and myocardial infarction at a young age. Thromb Haemost 71:707–712, 1994.
5. Wun T-C, Kretzmer KK, Girard TJ, Miletich JP, Broze G Jr: Cloning and characterization of a cDNA coding for the lipoprotein-associated coagulation inhibitor show that it consists of three tandem Kunitz-type inhibitory domains. J Biol Chem 263:6001–6004, 1988.
6. Broze G, Girard TJ, Novotny WF: The lipoprotein-associated coagulation inhibitor. In Colter BS (ed): ''Progress in Haemostasis and Thrombosis.'' vol. 10. Philadelphia: WB Saunders, 1990, pp 243–268.
7. Rapaport SI: The extrinsic pathway inhibitor, a regulator of tissue factor-dependent blood coagulation. Thromb Haemost 66:6–15, 1991.
8. Novotny WF, Brown SG, Mieltich JP, Rader DJ, Broze GJ: Plasma antigen levels of the lipoprotein-associated coagulation inhibitor in patient samples. Blood 78:387–393, 1991.
9. Lindahl AK, Jacobsen PB, Sandset PM, Abildgaard U: Tissue factor pathway inhibitor with high anticoagulant activity is increased in postheparin plasma and in plasma from cancer patients. Blood Coag Fibr 2:713–721, 1991.
10. Brandzaeg P, Sandset PM, Joo GB, Obstebo R, Abildgaard U, Kierulf P: The quantitative association of plasma endotoxin, antithrombin, protein C, extrinsic pathway inhibitor and fibrinopeptide A in systemic meningococcal disease. Thromb Res 55:459–470, 1989.
11. Kobayashi M, Wada H, Wakita Y, Shimura M, Nakase T, Hiyoyama K, Nagaya S, Minami N, Nakano T, Shiku H: Decreased plasma tissue factor pathway inhibitor levels in patients with thrombotic thrombocytopenic purpura. Thromb Haemost 73:10–14, 1995.
12. Valentin S, Nordfang C, Bregengard C, Wildgoose P: Evidence that the C-terminus of tissue factor pathway inhibitor (TFPI) is essential for its in vitro and in vivo interaction with lipoproteins. Blood Coag Fibr 4:713–720, 1993.
13. Abumiya T, Enjyoji K, Kokawa T, Kamikubo Y, Kato H: An antitissue factor pathway inhibitor (TFPI) monoclonal antibody recognized the third kunitz domain (K3) of free-form TFPI but not lipoprotein-associated forms in plasma. J Biochem 118:178–182, 1995.
14. Kokawa T, Enjyoji K, Kumeda K, Kamikubo Y, Harada-shiba M, Koh H, Tsushima M, Yamamoto A, Kato H: Measurement of free form of tissue factor pathway inhibitor (TFPI) antigen in hyperlipidemia: Relationship between free form and endothelial cell-associated forms of TFPI. Arterioscl Thromb Vasc Biol 16:802–808, 1996.
15. Kamei S, Kamikubo Y, Hamuro T, Fujimoto H, Ishihara M, Yonemura H, Miyamoto S, Funatsu A, Enjyoji K, Abumiya T, Miyata T, Kato H: Amino acid sequence and inhibitory activity of rhesus monkey tissue factor pathway inhibitor (TFPI): Comparison with human TFPI. J Biochem 115:708–714, 1994.
16. Kokawa T, Abumiya T, Kimura T, Harada-Siba M, Koh H, Ysushima M, Yamamoto A, Kato H: Tissue factor pathway inhibitor (TFPI) activity in human plasma: Measurement of lipoprotein-associated and free forms in hyperlipidemia. Arterioscl Thromb Vasc Biol 15:504–510, 1995.
17. Wada H, Minamikawa K, Wakita Y, Nakase T, Kaneko T, Ohiwa M, Tamaki S, Deguchi K, Shirakawa S, Hayashi T, Suzuki K: Increased vascular endothelial cell markers in patients with disseminated intravascular coagulation. Am J Hematol 44:85–88, 1993.
18. Shimura M, Wada H, Wakita Y, Nakase T, Hiyoyama K, Nagaya S, Mori Y, Shiku H: Plasma tissue factor and tissue factor pathway inhibitor levels in patients with disseminated intravascular coagulation. Am J Hematol 52:165–170, 1996.
19. Hoppensteadt DA, Walenga JM, Fasanella, A, Jeske W, Fareed J: TFPI antigen levels in normal human volunteers after intravenous and subcutaneous administration of unfractionated heparin and a low molecular weight heparin. Thromb Res 77:175–185, 1995.